GELATION OF MILK SOLIDS BY ORTHOPHOSPHATE

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ABSTRACT

The gelation of milk solids dispersed in high concentrations of orthophosphate was studied by following changes in viscosity of the solutions. The rate of the gelation process was found dependent on the concentration of phosphate, calcium, and caseinate complex. Also, the rate depends on pH, temperature, agitation, and the identity of the phosphate counterion. Below certain critical concentrations, polyphosphates retard gelation. Metal ions also retard gelation but have no critical concentration limits.

Viscosity data indicate that the gelation process consists of two phases: An initial, null phase characterized by little change in the viscosity and a second phase characterized by a rapid change in the viscosity. Although a high concentration of phosphate provides the driving force for the gelation process, calcium is also required. During gelation $CaHPO_4$ may possibly be bound at sites in the interior of the caseinate particles.

In a study of the effect of added phosphates in preventing gelation of sterile milk concentrates, Leviton and Pallansch (4) found that added orthophosphate hastened the onset of gelation relative to the control. Wilson et al. (7), in a somewhat similar investigation, found the same result. The occurrence of gelation in sterile concentrated milk products, and the hastening of such gelation when orthophosphate is added, point to the possibility of orthophosphate as the cause of gelation in concentrated milk products (2).

Rose and Tessier (5) have reported that orthophosphate at about 100 mM/liter and potassium chloride at 1 M/liter caused the gelation of milk at room temperature in a matter of hours. In the investigation reported here, the phosphate-induced gelation of the caseinate system of milk has been re-examined and more extensively characterized in terms of the factors which affect it.

METHODS AND MATERIALS

Changes in viscosity reflecting the development of gel structure in solutions of milk solids were measured, using an Ubbelohde viscometer having a delivery time for distilled water of 104.8 sec at 25 C. Solutions were filtered twice through a cotton plug and immediately poured into the viscometer, which was then tilted from the vertical to near horizontal three times prior to each measurement. The flow-through time of the solution was measured to 1/10 sec at

5-min intervals from the time the solution was made 0.20 M phosphate.

Solutions containing elevated levels of calcium were prepared by dissolving milk solids in water containing the desired level of calcium, as calcium chloride. Where required, the calcium concentration of solutions of milk solids was reduced by addition of a calculated amount of neutral M/2 $K_2C_2O_4$ solution. Precipitated CaC_2O_4 was removed by centrifuging at 600 × g for 10 min at room temperature. Both types of solution were allowed to stand overnight before phosphate additions.

The effect of urea on gelation by phosphate was studied in systems made by dissolving milk solids in 4 M urea solutions prior to phosphate addition.

Nitrogen determinations were made by a standard micro-Kjeldahl procedure (1).

Calcium was determined by back titration after addition of excess ethylene-diamine-tetra-acetate (8).

A Spinco Model L centrifuge was used where indicated.[1]

Nonfat milk solids used in this study were taken from a single lot of spray-dried material prepared from pasteurized skim, using conventional techniques.

All salts used were reagent grade. The hexametaphosphate used was obtained from Eimer and Amend Company.[1] Cyclophos was a Monsanto product.[1]

[1] Reference to certain products or companies does not imply an endorsement by the Department over others not mentioned.

The 0.2 M phosphate buffer, pH 6.85, was made by mixing 0.1 M each of K_2HPO_4 and KH_2PO_4 in sufficient water to make 1 liter volume. Where necessary, a pulverized mixture of the phosphate salts was added directly to the solution of milk solids to obtain the desired phosphate concentration.

RESULTS

Change in flow-through time with time in systems prepared by dispersing 6% SNF in 0.2 M phosphate buffer pH 6.85, and adding the equivalent amount of phosphate directly to a 6% SNF solution is shown in Figure 1. The observed increases in flow-through time are equivalent to increases in viscosity. From Figure 1 it can be seen that equilibration of the milk solids with water prior to phosphate addition increases the rate of observed viscosity increase. Both curves have the same general shape and consist essentially of two parts: a) a null phase in which there is little or no change in viscosity; b) an interaction phase characterized by a rapid increase in viscosity leading to gelation.

A semilog plot of the data taken between points representing flow-through times of 10 to 100 sec, Figure 2, reveals that the changes in the interaction phase are exponential in character. The slope of the straight lines obtained is proportional to the rate of the interaction. The lines in Figure 2 are described by the equation:

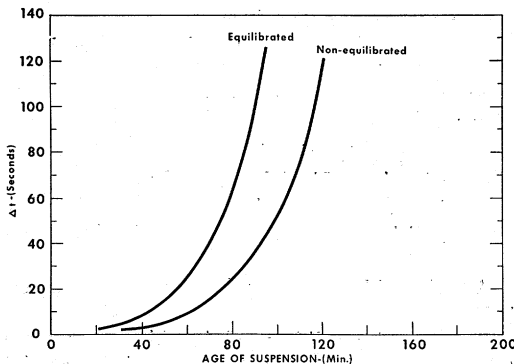$$ln\, \Delta\, t = K\, \Theta + C$$

FIG. 1. Rate of gelation of 6% SNF in 0.20 M phosphate (K$^+$ counterions) buffer, pH 6.85 at 25 C. The equilibrated solution consists of SNF reconstituted in water and left standing at room temperature (ca. 20 C) overnight before being made to 0.20 M with phosphate. The unequilibrated solution consists of SNF dispersed directly into 0.20 M phosphate buffer.

Age of suspension is measured from the time it is made 0.20 M phosphate. Δt is the increase in flow time of the solutions in the viscometer at a given age of the suspension of SNF.
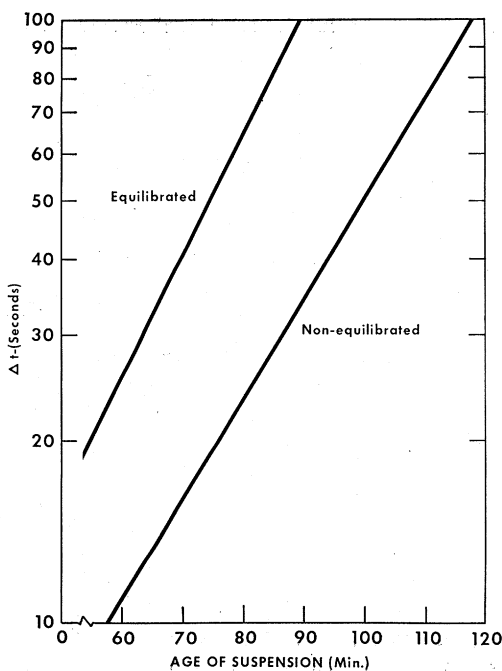

FIG. 2. Data of Figure 1 for $10 \leqslant \Delta t \leqslant 100$ sec plotted on semilog coordinates.

where Θ is the age of the suspension in min and K and C are constants.

During the course of making these observations, it was seen that the increase in Δt of the solution was accompanied by a decrease in opacity.

It was also noted that the agitation the solution encountered during the course of making the viscosity measurements approximately halved the time required to achieve gelation in a quiescent system.

Rate of gelation was also found to be directly dependent upon the concentration of the phosphate of the system, as shown in Figure 3.
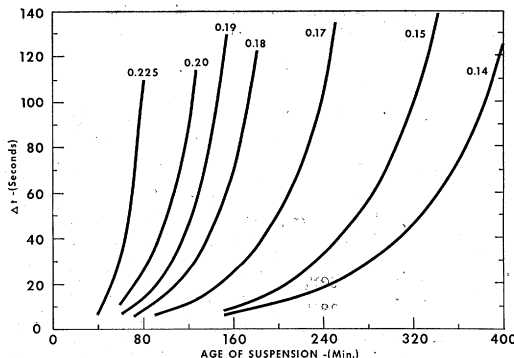

FIG. 3. Rate of gelation of 6% SNF at different concentrations of phosphate (K$^+$ counterions) at pH 6.85 and 25 C.

Here it is evident that increasing levels of phosphate increase gelation rates and also markedly reduce the duration of the null period.

Further investigation revealed not only that the phosphate radical is essential in the gelation process, since sulphates, carbonates, and chlorides did not produce this effect, but also that the nature of the counterion must be considered. Figure 4 graphically demonstrates that
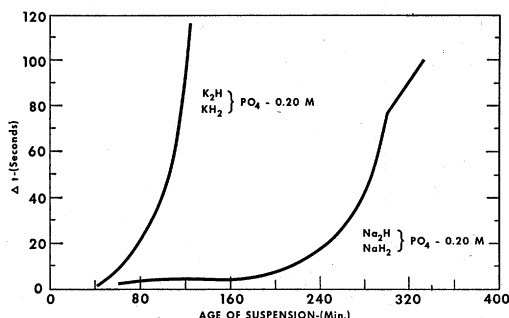
FIG. 4. Effect of the identity of the phosphate counterion on rate of gelation of 6% SNF in 0.20 M phosphate, pH 6.85 at 25 C.

gelation occurs much more slowly in a solution of milk solids containing 0.2 M mixed sodium phosphates, than in a solution containing the equivalent concentration of potassium phosphates. This is due primarily to an extension of the null period in the presence of sodium.

The concentration of calcium ion in the system also influences the rate of gelation, as shown in Figure 5. Higher levels of calcium shorten the null period and increase the rate of gelation in the interaction phase. When calcium is completely removed from milk by oxalate treatment, no viscosity increase was observed in systems containing SNF and 0.20 M phosphate. Figure 5 also shows the rate of gelation of a system prepared by dialyzing calcium-free SNF to equilibrium against milk. Here the duration of the null period was found to be longer than anticipated from the measured calcium concentration of the equilibrated solution.

Increasing the concentration of SNF in the high phosphate systems also influenced gelation, as shown in Figure 6. The null period decreased as the SNF content was increased. An increase in gelation rate in the interaction phase, with increase in SNF, also occurred, as shown in Figure 7. The points describe a straight line, except at the highest SNF concentration.

Similar, but less marked, decreases in the null period and increases in gelation rate were
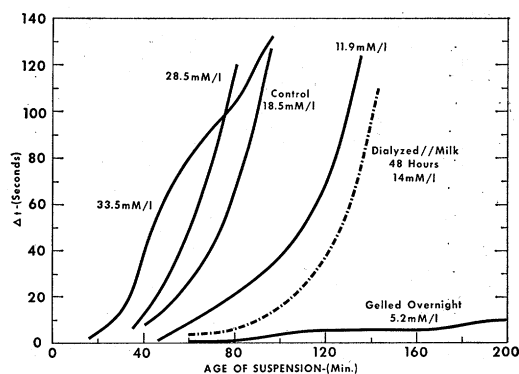
FIG. 5. Effect of different concentrations of calcium on the gelation rate. Concentration of milk solids is 6% in 0.20 M phosphate (K+ counterions) buffer at pH 6.85, temp 25 C. The control contained 18.5 mM/liter of calcium. Graphs to the left of the control are of milk solids containing added calcium.

Graphs to the right of the control are of milk solids from which calcium has been removed to the concentration indicated. Milk indicated as dialyzed milk had calcium completely removed with oxalate prior to dialysis. This decalcified milk was then dialyzed against a large excess of milk for 48 hr.
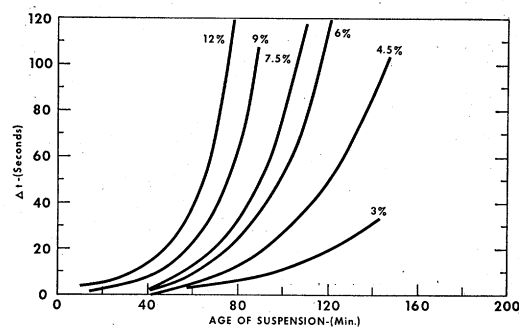
FIG. 6. Effect of different concentrations of milk solids-not-fat on gelation rate, temp 25 C; 0.20 M phosphate (K+ counterions); pH 6.85.

also observed on addition of various monovalent cations to systems containing SNF and 0.20 M orthophosphate, as shown in Figure 8.

Opposite effects were observed on addition of some divalent cations, hexametaphosphate (HMP), and Cyclophos (P), as shown in Figure 9. During the course of the experimental work, it was observed that HMP did not retard gelation in systems containing 4 M urea in addition to SNF and 0.20 M orthophosphate. The presence of urea did not influence the stabilizing effect of the metal ions in similar systems.

Gelation rates were also found to be pH dependent, as shown in Figure 10. Maximum gelation rates were observed at neutrality. Ge-
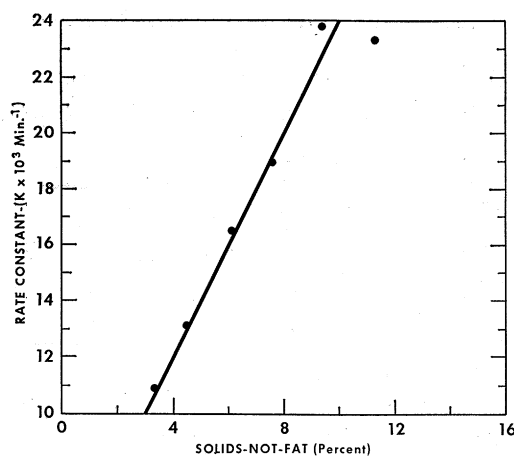
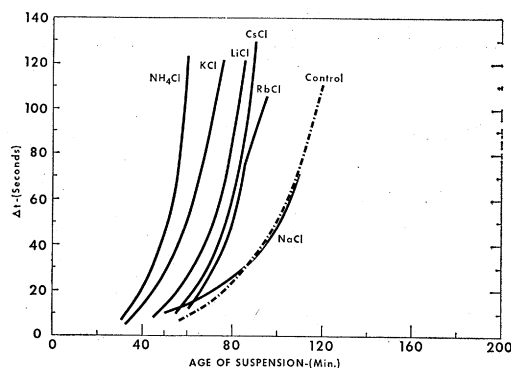FIG. 7. Dependence of the rate constant on the concentration of the milk solids.

FIG. 8. Effect of different added alkali metal ions and NH_4^+ ions on rate of gelation of 6% milk solids. 0.20 M phosphate (K^+ counterions) buffer, pH 6.85 at 25 C. The concentration of the added ion is 0.2 M.
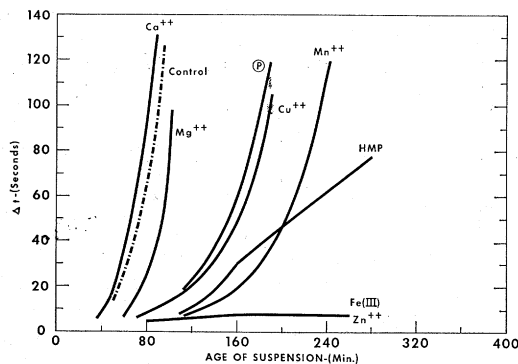
FIG. 9. Effect of different additives on gelation rate. All additives were equilibrated in 6% SNF overnight at room temperature. Age of suspension measured from time of addition of 0.20 M phosphate (K^+ counterion), pH 6.85. Viscosity measured at 25 C. All metals are at a concentration of 3 mM/liter. HMP is at 1.3 mM/liter (avg of 7 P atoms per chain). Cyclophos—P—is at 1.5 mM/liter.
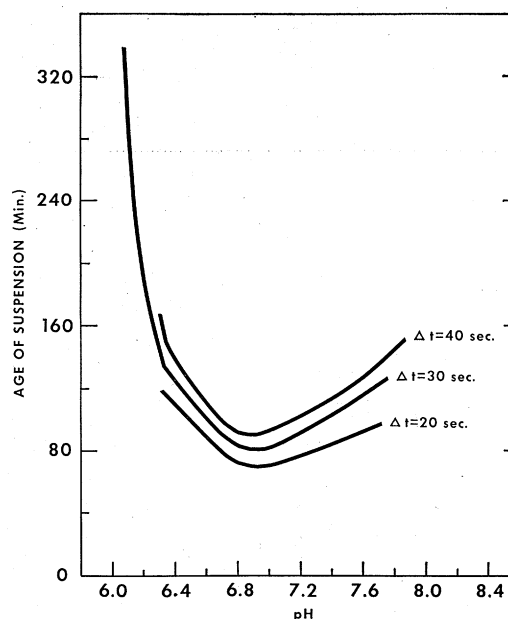
FIG. 10. Effect of pH on gelation rate of 6% SNF dispersed in 0.20 M phosphate (K^+ counterions) buffer at 25 C. Age of the suspension (in minutes) is plotted vs. viscometer flow-time increments of 20, 30, and 40 sec.

lation rates fell off more rapidly as the pH of the systems moved toward lower values.

The gelation rate could also be controlled by temperature, as shown in Figure 11. Here the decrease in null period and increase in gelation rate with increasing temperature is shown.

As the temperature of the system is increased, the difference in the duration of the null period observed in systems containing either Na^+ or K^+ becomes smaller. This effect is shown graphically in Figure 12.

Analytical data pertaining to change in the distribution of nitrogen and calcium in SNF
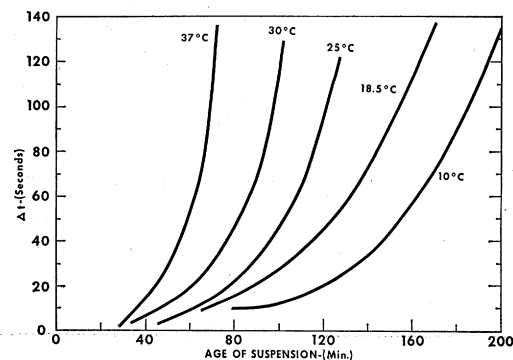
FIG. 11. Effect of temperature on rate of gelation of 6% SNF dispersed in 0.20 M phosphate (K^+ counterions) at pH 6.85.
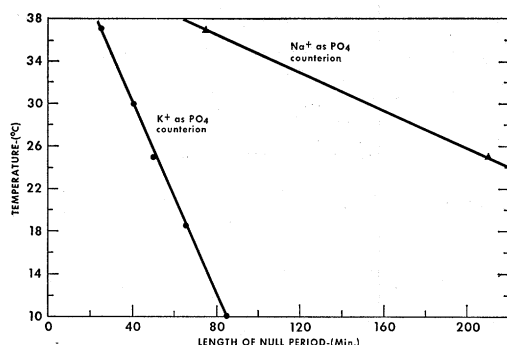
FIG. 12. Temperature dependency of the null period. The effect of the identity of the counterion on the temperature effect is shown for Na$^+$ and K$^+$ ions.

systems during gelation are presented in Table 1. These data show a general increase in the calcium-to-nitrogen ratio in the sedimentable fraction of SNF as gelation occurs.

surface of the caseinate particle possesses a structure similar to a cell membrane in its ability to differentiate between Na$^+$ and K$^+$ ions. Examples of the latter type of selective diffusion are well-documented in the literature of cellular physiology (3, 6).

When the amount of phosphate diffusing into the interior of the caseinate particle reaches a critical level, structural changes occur which change the optical properties of the system, by decreasing opacity, and the modified particles begin to interact to form a gel. This structural change in the caseinate system to produce an interacting species marks the end of the null phase and the start of the interaction phase.

Both the duration of the null period and the rate of subsequent interaction are influenced by many of the same physical and chemical properties of the systems in which they occur. However, the influence of the counterion asso-

TABLE 1

Distribution of nitrogen and calcium in solution of normal and gelled milk solids

Per cent total solids	3.36	4.53	6.14	7.58	9.43	11.3
Total nitrogen (%)	.183	.236	.333	.407	.517	.604
Per cent total nitrogen as centrifugable nitrogen [a]	67.2	69.0	72.1	71.7	72.8	73.2
Per cent total nitrogen as gel nitrogen [b]	31.2	26.3	32.4	35.1	38.5	33.1
Total calcium (mM/liter)	10.75	13.75	18.5	23.5	29.5	34.5
Per cent total calcium as centrifugable Ca [a]	55.8	60.0	62.2	65.9	67.8	67.4
Per cent total calcium in gel structure [b]	84.8	87.3	90.5	94.0	91.2	92.7

[a] Centrifugation at 105,000 × g (avg) for 30 min. Top two-thirds of supernatant was removed from tube for analysis. Solution did not contain added phosphate at time of analysis.
[b] Centrifugation at 600 × g (avg) for 5 min. Total supernatant was poured from tube for analysis. Gel broken up and centrifuged 30 min after appearance of firm gel.

DISCUSSION

From results presented, it can be visualized that the gelation of SNF by high levels of orthophosphate is a two-step process, consisting of a null phase characterized by its duration and an interaction phase characterized by its rate.

During the null phase, penetration of the casein aggregate by phosphate can be considered to occur. Rate of penetration is greatly influenced by the nature of the counterion and the presence of other salts in the system.

The difference in effect of Na$^+$ and K$^+$ counterions on the gelation rate may be a consequence of a difference in the diffusion rate of these ions paired with $HPO_4^=$ when moving into the interior of the caseinate particle. From the observed loss of this discriminating effect in systems containing 4 M urea and at high temperatures it is possible to consider that the

ciated with the phosphate seems operative only during the null phase. This observation is substantiated by the similarity of the slopes of the lines in Figure 13, where the logarithms of the rate constants of the interaction phases at different temperatures are plotted against the reciprocal of the absolute temperature.

From the slope of the line pertaining to potassium phosphate-induced gelation, as shown in Figure 13, and the Arrhenius equation, the free energy of activation is calculated to be 8.5 KCal. From the expressions for the activated complex (3), the following values for the free energy increment (ΔF) were calculated:

$$\Delta F^* = \Delta H^* - T \Delta S^*$$

$$22.4 \text{ KCal} = 7.9 \text{ KCal} - 298 \ (-48.5 \text{ eu})$$

The large decrease in entropy (ΔS) implies an increase in the order of the system, which
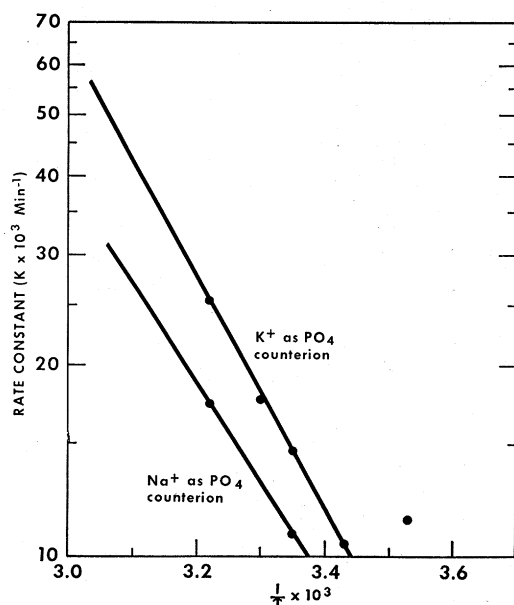
FIG. 13. Effect of the identity of the counterion of phosphate on the interaction phase at different temperatures. Plot of log K vs. $1/T$ where K is the rate constant (min^{-1}) for the interaction phase and T is the absolute temperature.

could arise from the binding of some constituent to the casein complex. If the bound ligand is considered to be $CaHPO_4$, then in a cooperative binding process

$$\frac{d(CaHPO_4)_{bound}}{d\Theta} = K(CaHPO_4)_{bound}$$

If $(CaHPO_4)_{bound} = k_1\Delta t$, then

$$\frac{d\Delta t}{d\Theta} = \frac{K k_1 \Delta t}{k_1} = K\Delta t$$

or: $ln \Delta t = K\Theta + C$

where Θ is the age of the suspension in minutes. This equation describes the curves shown in Figure 2, as already stated. Further consideration of the dependence of the rate of binding on the concentration of $CaHPO_4$, which is dependent upon the total calcium content of the system, would explain the observed increases in gelation rate with increased Ca^{++} ion concentrations, as presented in Figure 5.

The role of bound calcium in gel formation can be further deduced from a consideration of the effect of pH and gelation rates. Below pH 7.0 the binding of calcium by casein has been shown to drop off rapidly with decreasing pH (9). This can be accounted for in part by the equilibrium

$$HPO_4^= + H^+ \rightleftharpoons H_2PO_4^-$$
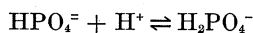
The pK of this dissociation is about 6.6, so that below this value the amount of $HPO_4^=$, of $CaHPO_4$ and, consequently, the gelation rate should drop off exponentially.

$$\log \frac{H_2PO_4^-}{HPO_4^=} = pK - pH$$

In Figure 10 this is seen to be the case. Above pH 7.0 it is probable that gelation rates fall off, due to increased electrostatic repulsion of the casein particles. At still higher values of pH, calcium exists as its hydroxide and may no longer be bound.

From observations of the gel-retarding properties of hexametaphosphate (HMP) and heavy metal ions in systems containing 4 M urea, it can be concluded that HMP is effective only in increasing the length of the null period. The metal ions actually decrease the interaction rate. Further, HMP is unique in the fact that if present in excess of a critical concentration (1.4 g/100 g SNF) it completely eliminates the null period and phosphate-induced viscosity increases are immediately observed. Stabilizing metal ions exhibit no such critical concentration range.

Although the data presented in this paper pertain to phosphate-induced gelation in systems containing reconstituted SNF, it has been determined that fresh milk, concentrated milk, and milk solids dried by different procedures all undergo gelation by high levels of orthophosphate. It is of interest that the reported gelation is similar to observed gelation in concentrated milk sterilized by high temperature–short time methods in the following respects:

(1) The rate of gelation is increased by agitation.

(2) The rate of gelation increases with increasing temperature.

(3) Rate of gelation is pH-dependent.

(4) Gelation is retarded by hexametaphosphate and some metal ions.

(5) There is a critical concentration of hexametaphosphate above which gelation rates are actually increased.

The implication of these observed similarities is furnishing the basis for further research in our laboratory.

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